

Size-dependent peroxidase-like catalytic activity of Fe₃O₄ nanoparticles

Fang Fang Peng^{a,b,c}, Yu Zhang^{a,b}, Ning Gu^{a,b,*}

^a State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China

^b Jiangsu Laboratory for Biomaterials and Devices, Southeast University, Nanjing 210096, China

^c School of Chemistry and Chemical Engineering, Southeast University, Nanjing 210096, China

Received 25 December 2007

Abstract

Peroxidase-like catalytic properties of Fe₃O₄ nanoparticles (NPs) with three different sizes, synthesized by chemical coprecipitation and sol–gel methods, were investigated by UV–vis spectrum analysis. By comparing Fe₃O₄ NPs with average diameters of 11, 20, and 150 nm, we found that the catalytic activity increases with the reduced nanoparticle size. The electrochemical method to characterize the catalytic activity of Fe₃O₄ NPs using the response currents of the reaction product and substrate was also developed. © 2008 Ning Gu. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.

Keywords: Fe₃O₄ nanoparticles; Peroxidase-like catalytic activity; Electrochemistry

Magnetic nanoparticles (NPs) are leading candidates for medical applications because of their unique multifunctional properties. Indeed, the same magnetic nanocrystal may serve as a contrast agent for magnetic resonance imaging [1], as a vector for magnetic guidance [2] or gene detection [3], and also as a nanosource of heat for hyperthermia [4]. In industrial treatment of wastewater, magnetic NPs show hydroxylation activity and are often used as a kind of catalyst for the removal of phenolic solution [5]. Recent promising work in this field is the immunoassay using the dual functionality of the Fe₃O₄ magnetic NPs as a peroxidase mimic catalyst and magnetic separator [6], which has potential applications in medicine, biotechnology and environmental chemistry.

Here, we focus on peroxidase-like catalytic properties of Fe₃O₄ NPs. The Fe₃O₄ NPs with three different sizes (11, 20, and 150 nm), synthesized by chemical coprecipitation and sol–gel methods, can all catalyze the reaction of the typical peroxidase substrate 3,3',5',5'-tetramethylbenzidine (TMB) in the presence of H₂O₂. And interestingly, as the sample size reduces, the catalytic activity increases. At the same time, the electrochemical method to characterize the catalytic activity of Fe₃O₄ NPs using the response currents of the reaction product and substrate was also developed.

1. Experimental

Fe₃O₄ NPs with diameters of approximately 11 and 20 nm were prepared according to the co-precipitation method of Molday [7] and 150 nm Fe₃O₄ NPs were synthesized by the sol–gel method [8]. The NPs appeared spherical and

* Corresponding author at: State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China.

E-mail address: guning@seu.edu.cn (N. Gu).

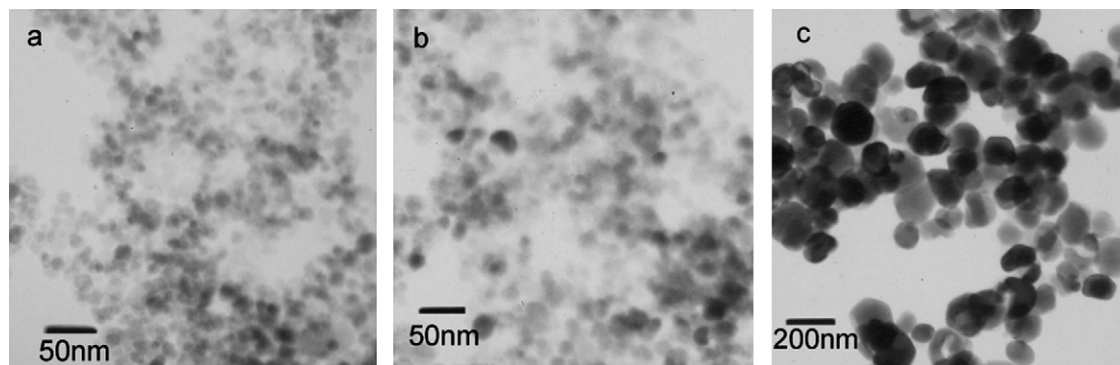


Fig. 1. TEM images of Fe_3O_4 NPs with different average diameters: (a) 11 nm, (b) 20 nm, and (c) 150 nm.

homogeneous (Fig. 1). All the electrochemical voltammetry measurements were carried out on CHI660 electrochemical workstation (China) by three-electrode system with a glassy carbon electrode (GCE, $D = 3$ mm) as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. $80\ \mu\text{L}$ H_2O_2 (1 mol/L) and $800\ \mu\text{L}$ TMB (1 mg/mL) were added to 20 mL of phosphate buffer solution (pH 7.0) with and without $100\ \mu\text{L}$ Fe_3O_4 NPs. After different reaction time, the product was analyzed on the electrochemical workstation.

2. Results and discussion

The Fe_3O_4 NPs can catalyze the oxidation of the substrate TMB by H_2O_2 just like horseradish peroxidase (HRP) and the catalytic ability is size-dependent. As shown in Fig. 2a, the catalytic activity of Fe_3O_4 NPs with sizes of 11, 20, and 150 nm was compared by the absorbance variation of the product at 650 nm in UV–vis spectra. The results show that all the three catalysts are active for the substrate, and the activities for the reaction vary substantially in the order $11\ \text{nm} > 20\ \text{nm} > 150\ \text{nm}$. It implies that with the reduced sample size, the catalytic activity increases. This phenomenon may be due to the small size sample having more active sites and larger specific surface area, which lead to more efficient contact with the substrate. In addition, typical Michaelis–Menten curve was observed for Fe_3O_4 NPs (Fig. 2b). The data was fitted to the Michaelis–Menten model to obtain the parameter apparent K_m . We found that the K_m value of Fe_3O_4 NPs with TMB as the substrate was 0.27, which was lower than that of HRP, 0.4. It suggested that Fe_3O_4 NPs have a higher affinity for TMB than HRP and may be used as a hopeful peroxidase mimic.

The electrochemical method to characterize the catalytic activity of Fe_3O_4 NPs was developed. As shown in Fig. 3, the cyclic voltammograms were recorded from -0.2 to 0.6 V at the scan rate of 0.1 V/s, and three pairs of redox peaks

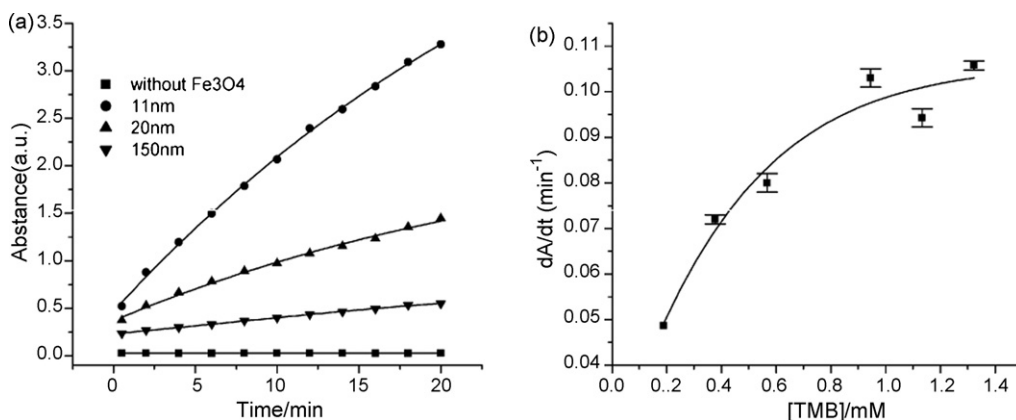


Fig. 2. Size-dependent peroxidase-like catalytic activity of Fe_3O_4 NPs. (a) Time-dependent absorbance curves of the product in the absence (■) and presence of Fe_3O_4 NPs with average diameters of 11 (●), 20 (▲) and 150 nm (▼) under the optimized experimental conditions; (b) steady-state kinetic assay of Fe_3O_4 NPs with TMB as the substrate.

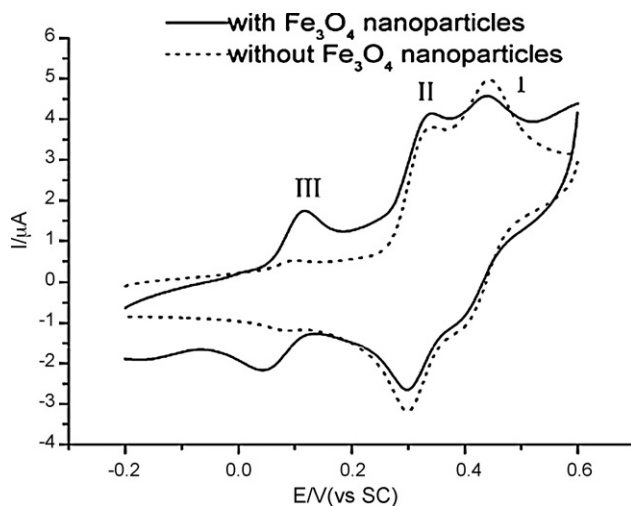
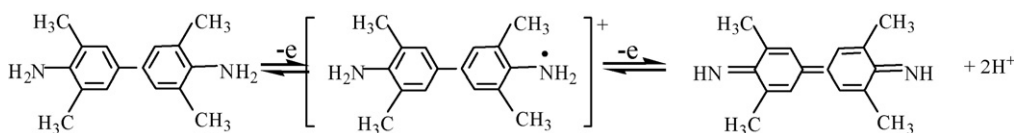


Fig. 3. The cyclic voltammograms of the reaction system without (...) and with (—) Fe_3O_4 NPs after 2 h reaction time.



Scheme 1. The schematic reaction course of the substrate TMB.

I–III were obtained after 2 h reaction time. The two pairs of peaks at the higher potential are due to the substrate, TMB, which underwent two successive one-electron processes [9]. And the pair of redox peaks III at the lower potential is assigned to reaction product, from which we found that the conversion rate of product was obviously enhanced by Fe_3O_4 NPs. Scheme 1 shows the reaction course of TMB, corresponding with the electrochemical result, which can also be approved by UV–vis analytical method.

Fig. 4 shows the square-wave voltammetric behavior of reaction system with and without Fe_3O_4 NPs after different reaction time. The response current of reaction product appears at 0.08 V and the two peaks at 0.42 and 0.31 V are corresponded to the substrate, TMB. The oxidation of TMB by H_2O_2 in the system without Fe_3O_4 NPs was so slow that there was no obvious change of the current signal during 2 h reaction time (Fig. 4a). When Fe_3O_4 NPs were added to the mixture, as shown in Fig. 4b, the response current of catalytic reaction product increased rapidly with the current of the substrate decreasing. 2 h later, the current of the product increased to $1.8 \mu\text{A}$, which was even larger than that in the

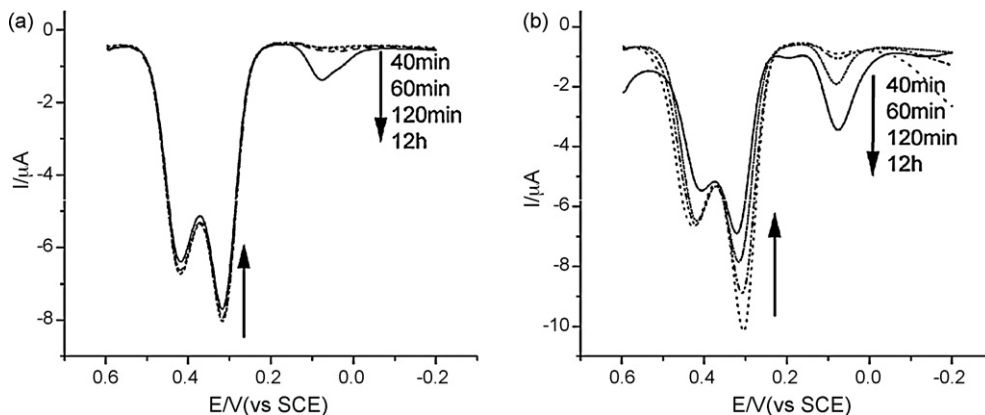


Fig. 4. Square-wave voltammetric behavior of reaction system without (a) and with (b) Fe_3O_4 NPs after different reaction time.

system without Fe_3O_4 NPs after 12 h reaction time. These results indicate that the Fe_3O_4 NPs, like peroxidase, can effectively catalyze the reaction of the substrate TMB in the presence of H_2O_2 .

In conclusion, as a new kind of enzyme mimics, Fe_3O_4 NPs not only have a lot of advantages such as moderate cost, ease of preparation and long-time stability comparing to natural enzymes but also they possess more important magnetic characteristics just as discussed above. The peroxidase-like catalytic activity of Fe_3O_4 NPs and its size-dependence will extend the application of the materials in biological and chemical field. The electrochemical method to characterize the catalytic activity of Fe_3O_4 NPs using the response currents of the product and substrate may also stimulate the exploration of applications of these NPs.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 90406023 and 60571031) and National Important Science Research Program of China (Nos. 2006CB933206 and 2006CB705606).

References

- [1] J. Wan, W. Cai, X. Meng, et al. *Chem. Commun.* 47 (2007) 5004.
- [2] T.K. Jain, M.A. Morales, S.K. Sahoo, et al. *Mol. Pharm.* 2 (2005) 194.
- [3] L. Josephson, J.M. Perez, R. Weissleder, *Angew. Chem. Int. Ed.* 40 (2001) 3204.
- [4] F. Sonvico, S. Mornet, S. Vasseur, et al. *Bioconjug. Chem.* 16 (2005) 1181.
- [5] D.Y. Wang, Z.Q. Liu, F.Q. Liu, *Appl. Catal. A* 174 (1998) 25.
- [6] L. Gao, J. Zhuang, L. Nie, et al. *Nat. Nanotechnol.* 2 (2007) 577.
- [7] R.S. Molday, US Patent 452 (1984) 773.
- [8] T. Sugimoto, E. Matijevic, *J. Colloid Interface Sci.* 74 (1980) 227.
- [9] Z. He, W. Jin, *Anal. Biochem.* 313 (2003) 34.